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Bacterial *Peptide deformylase* inhibition of cyano substituted biaryl analogs: Synthesis, in vitro biological evaluation, molecular docking study and in silico ADME prediction

Firoz A. Kalam Khan [a], Rajendra H. Patil [b], Devanand B. Shinde [c], Jaiprakash N. Sangshetti [a,*]

[a] *Y.B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Aurangabad 431001, M.S., India*
[b] *Department of Biotechnology, Savitribai Phule Pune University, Pune 411007, M.S., India*
[c] *Shivaji University, Vidyanagar, Kolhapur 416 004, M.S., India*

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ABSTRACT

Herein, we report the synthesis and screening of cyano substituted biaryl analogs **5**(**a**–**m**) as *Peptide deformylase* (PDF) enzyme inhibitors. The compounds **5a** (IC_{50} value = 13.16 μM), **5d** (IC_{50} value = 15.66 μM) and **5j** (IC_{50} value = 19.16 μM) had shown good PDF inhibition activity. The compounds **5a** (MIC range = 11.00–15.83 μg/mL), **5b** (MIC range = 23.75–28.50 μg/mL) and **5j** (MIC range = 7.66–16.91 μg/mL) had also shown potent antibacterial activity when compared with ciprofloxacin (MIC range = 25–50 μg/mL). Thus, the active derivatives were not only potent PDF inhibitors but also efficient antibacterial agents. In order to gain more insight on the binding mode of the compounds with PDF, the synthesized compounds **5**(**a**–**m**) were docked against PDF enzyme of *Escherichia coli* and compounds exhibited good binding properties. In silico ADME properties of synthesized compounds were also analyzed and showed potential to develop as good oral drug candidates.

1. Introduction

An infectious disease caused by microorganisms affects millions of people worldwide and cause millions of death each year. Also, increasing antibacterial resistance poses a severe threat to human health.[1–3] As consequence, there is an urgent demand to identify new antibiotics that do not share the targets of existing antibacterial drugs. Many novel and potentially useful targets are discovered by analysis of microbial genomes, but, so far, little has been achieved from these efforts.[4] One target that has not received much attention until recently is *Peptide deformylase* (PDF).[5] PDF has been a possible target that may fulfill all the criteria essential for good target to develop new antibacterial agents with novel mechanism of action.[6] The difference in protein synthesis between bacteria and mammalian cells stems from transformylation and deformylation of initiating methionine. The process for bacterial protein synthesis is initiated with *N*-formylmethionine, which is generated by transformylation of methionine. The *N*-formyl group of the polypeptide (emerges from ribosome after completion of elongation process) is removed by the sequential action of PDF.[7] The fact that the PDF is essential for producing the mature protein in bacterial provides a rational basis to choose it as a potential and novel target for antibacterial activity.

In the past few years, different classes of PDF inhibitor like, peptidic inhibitors, pseudopeptidic inhibitors and non-peptidic inhibitors as antibacterial agents have been reported.[8] The non-peptidic inhibitors like, biaryl acid analogs were developed by Merck Research Laboratories and evaluated as PDF inhibitor against *Escherichia coli* PDF. A representative structure for these biaryl acid analogs is shown in Figure 1. The biaryl acid analogs are composed of a 'head group' of aromatic rings, a biaryl group, and an acidic group on the biaryl B-ring. Structure–activity relationship (SAR) studies of biaryl acid analogs revealed that substitution at the head group, biaryl group, and the nature of acidic group all contributed to the inhibitory activity of the these compounds against PDF. The acidic group of these compounds may bind to the metal ion and instead interact with an amino acid residue within PDF active site much like the binding of the angiotensin II receptor. Two biaryl acid compounds **1** (IC_{50} = 3.9 μM) and **2** (IC_{50} = 22.8 μM) are presented in Figure 1. Barbara et al. explored groups like, carboxylate, tetrazole, amino and sulfonamide as acidic pharmacophore,[9] but they did not studied about the effect of cyano group as acidic pharmacophore on bacterial PDF enzyme inhibition. Based on these reports and to study the effect of cyano group as acidic pharmacophore, we, therefore, decided to explore the biaryl analogs

* Corresponding author. Tel.: +91 240 2381129.
E-mail address: jnsangshetti@rediffmail.com (J.N. Sangshetti).

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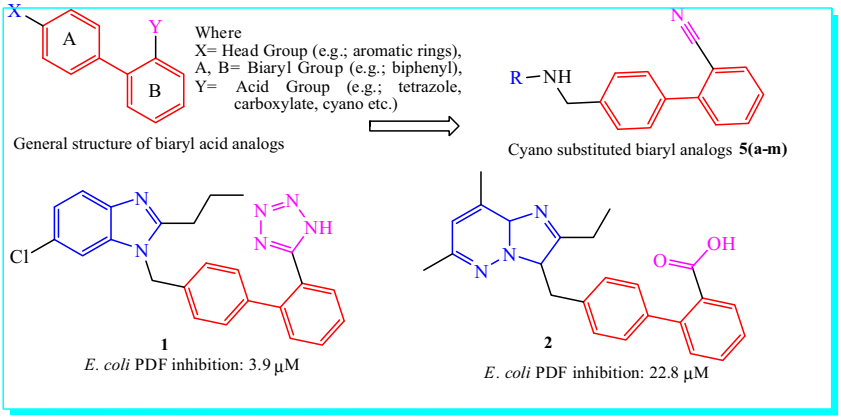


Figure 1. Design of cyano substituted biaryl analogs **5**(**a**–**m**).

containing cyano group as acid pharmacophore and study SAR by varying the head group (aromatic ring) for PDF inhibition activity.

Here, in continuous of our work on synthesis of bioactive molecules,[10–15] we report the design and synthesis of a series of cyano substituted biaryl analogs **5**(**a**–**m**), and the study of their effects on inhibition of *E. coli* PDF. The compounds were also evaluated for antibacterial against *Bacillus subtilis* and *E. coli*. To explore the underlying mechanisms of PDF inhibition, we docked synthesized compounds against *E. coli* PDF enzyme. We have also assessed the synthesized compounds for in silico ADME prediction and results showed that compounds could be exploited as oral drug candidate.

2. Results and discussion

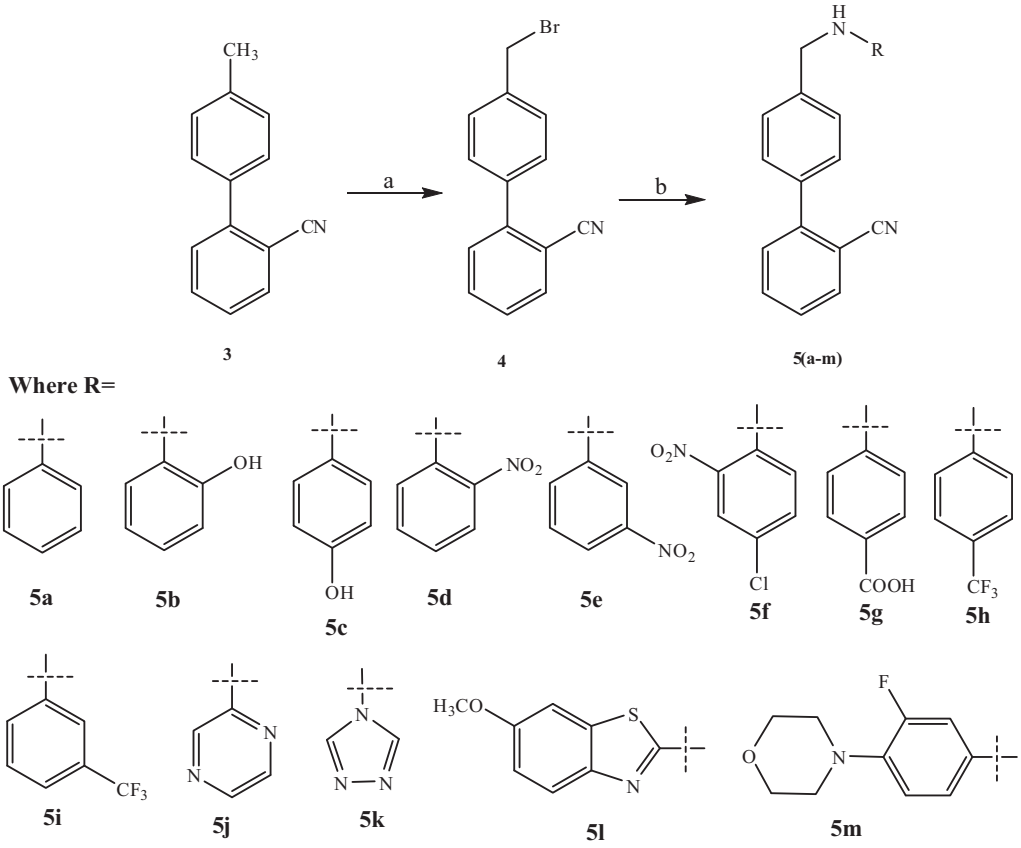
The synthetic approaches employed for synthesis of cyano substituted biaryl analogs **5**(**a**–**m**) are outlined in Scheme 1. The 4′-(bromomethyl)biphenyl-2-carbonitrile **4** was synthesized from commercially available 4′-methylbiphenyl-2-carbonitrile **3** in good yield (85%) according to published procedure.[16] Further, to expand the series, cyano substituted biaryl analogs **5**(**a**–**m**) were prepared reacting the compound **4** with various substituted aromatic/heterocyclic amines in *N*,*N*-dimethylformamide (DMF) using K_2CO_3 as catalyst. All the reactions proceeded well in 4–6 h to give products in very good yields (80–90%). The purity of the synthesized compounds was checked by TLC and melting points were determined in open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected. All synthesized derivatives **5**(**a**–**m**) were characterized using IR, ^{1}H NMR, ^{13}C NMR, Mass and elemental analysis and data suggested for proposed structures.

The *Peptide deformylase* enzyme was extracted from *E. coli* bacteria and stabilized using 5 mM $NiCl_2$. The synthesized cyano substituted biaryl analogs **5**(**a**–**m**) were evaluated for inhibition of *E. coli* PDF-Ni enzyme. The spectrophotometric method was employed for study of PDF inhibition activity. The IC_{50} value (concentration that decreased PDF by 50%) of synthesized compounds is presented in Table 1. The synthesized compounds **5**(**a**–**m**) had shown good (IC_{50} range = 13.16–51.16 μM) PDF inhibition activity. Compounds **5a** (IC_{50} = 13.16 μM), **5d** (IC_{50} = 15.66 μM) and **5j** (IC_{50} = 19.66 μM) were considered as most active *E. coli* PDF-Ni enzyme inhibitors. Compounds **5e** (IC_{50} = 25.00 μM), **5f** (IC_{50} = 28.25 μM), **5i** (IC_{50} = 20.91 μM) and 5 l (IC_{50} = 27.75 μM) had shown moderate PDF inhibition activity. Compounds **5b** (IC_{50} = 33.00 μM), **5c** (IC_{50} = 34.58 μM), **5g** (IC_{50} = 51.16 μM), **5h** (IC_{50} = 33.50 μM), **5k** (IC_{50} = 50.75 μM) and **5m** (IC_{50} = 42.50 μM) were found to be less active against *E. coli* PDF-Ni enzyme.

Structure–activity studies of cyano substituted biaryl analogs **5** (**a**–**m**) revealed that head group, biaryl group and acidic group (–*CN*) all contributed to inhibitory activity against PDF. The compounds **5**(**a**–**m**) showed varied PDF inhibition activity depending upon the various substituents present on phenyl ring (head group). Compound **5a** (IC_{50} = 13.16 μM) with *R* = *phenyl* showed most potent PDF inhibition activity among the synthesized compounds. The substitution of –*OH* group on phenyl ring **5b** (IC_{50} = 33.00 μM) and **5c** (IC_{50} = 34.58 μM) led to decrease in PDF inhibition activity by 2.5 fold. The introduction of *2-*NO_2 at phenyl ring **5d** (IC_{50} = 15.66 μM) led to increase in PDF inhibition activity and showed comparable activity as that of compound **5a**. On the other hand, introduction of *3-*NO_2 at phenyl ring **5e** (IC_{50} = 25.00 μM) led to decrease in PDF inhibition activity by 1.5 fold when compared with compound **5d**. Also, when *4-Cl* was introduced to 2-nitrophenyl ring, compound **5f** (IC_{50} = 28.25 μM) showed decrease in PDF inhibitory activity by 2 folds when compared with compound **5d**. The introduction of 4-COOH group on phenyl ring **5g** (IC_{50} = 51.16 μM) led to most inactive compound among the synthesized compounds. The replacement of *4-COOH* group with *4-*CF_3 on phenyl ring **5h** (IC_{50} = 33.50 μM) had increased the PDF inhibition activity. Further, when *4-*CF_3 group was replaced with *3-*CF_3 on phenyl ring **5i** (IC_{50} = 20.19 μM) led to increase in PDF inhibition activity by 1.5 fold when compared with compound **5h**. Thus, compounds **5d**, **5e**, **5f** and **5i** with electron withdrawing groups (except **5h**) like, –NO_2, –*Cl* and –CF_3 were more active than compounds **5b**, **5c** and **5g** with electron donating groups like, –*OH* and –*COOH* on phenyl ring (head group).

We have also analyzed the effect the some heterocyclic aromatic nucleus like, pyrazinyl, 1,2,4-triazolyl and 6-methoxybenzothiazolyl by replacing phenyl ring (head group) for PDF inhibition activity. Compound **5j** (IC_{50} = 19.16 μM) with *R* = pyrazinyl showed significant PDF inhibition activity when compared with most active compound **5a** (IC_{50} = 13.16 μM). When pyrazinyl ring was replaced with 1,2,4-triazolyl **5k** (IC_{50} = 50.75 μM) led to decrease in PDF inhibition activity by 2.5 folds. Further, compound **5l** (IC_{50} = 27.75 μM) with *R* = *6-methoxybenzothiazolyl* had shown improved PDF inhibitory activity by 2 folds when compared with compound **5k**. We also introduced 3-fluoro-4-morpholinophenyl (Linezolid core) as head group, but compound **5m** (IC_{50} = 42.50 μM) did not show any significant PDF inhibition activity.

The antibacterial activity was evaluated against one Gram-negative bacteria namely, *E. coli* (NCIM-2256) and one Gram-positive bacteria namely, *B. subtilis* (NCIM-2063) using ciprofloxacin as standard drug. Minimum inhibitory concentration (MIC) values were determined using standard agar method. Dimethyl sulfoxide was



Scheme 1. Synthesis of cyano substituted biaryl analogs **5**(**a**–**m**). Reagents: (a) NBS, H_2O_2, DCM, reflux; (b) amines, K_2CO_3, DMF, reflux.

Table 1
PDF enzyme inhibition and antibacterial activities of cyano substituted biaryl analogs **5**(**a**–**m**)

Entry	IC_{50} ± SEM (μM) *E. coli* PDF-Ni	MIC ± SEM (μg/mL)	
		E. coli	*B. subtilis*
5a	13.16 ± 1.18	15.83 ± 0.98	11.00 ± 0.52
5b	33.00 ± 2.75	39.58 ± 2.65	29.33 ± 1.75
5c	34.58 ± 1.91	33.58 ± 2.87	28.33 ± 1.36
5d	15.66 ± 0.87	28.50 ± 1.46	23.75 ± 0.88
5e	25.00 ± 1.68	40.91 ± 3.05	29.33 ± 1.13
5f	28.25 ± 2.53	45.58 ± 1.98	27.16 ± 1.15
5g	51.16 ± 4.54	32.58 ± 1.84	26.75 ± 1.50
5h	33.5 ± 1.66	35.50 ± 2.53	34.41 ± 1.42
5i	20.91 ± 1.45	42.50 ± 2.15	25.83 ± 1.25
5j	19.16 ± 1.88	16.91 ± 1.17	7.66 ± 0.24
5k	50.75 ± 1.67	36.50 ± 2.14	23.75 ± 1.20
5l	27.75 ± 2.56	35.25 ± 1.25	36.25 ± 2.56
5m	42.50 ± 1.71	37.33 ± 0.75	21.58 ± 0.84
Ciprofloxacin	ND	25.00 ± 0.95	50.00 ± 1.75

Experiments were performed in triplicates and compared to DMSO-treated controls; standard errors were all within 10% of the mean; ND: not done.

used as solvent control. MIC values of the tested compounds are presented in Table 1. Interestingly, our results demonstrated that most potent PDF inhibitors **5a** (MIC range = 11.00–15.83 μg/mL), **5d** (MIC range = 23.75–28.50 μg/mL) and **5j** (MIC range = 7.66–16.91 μg/mL) showed also a significantly potent antibacterial activity against *E. coli* and *B. subtilis* when compared with standard ciprofloxacin (MIC range = 25.00–50.00 μg/mL). Compounds **5a** (MIC = 15.83 μg/mL) and **5j** (MIC = 16.91 μg/mL) showed most potent antibacterial activity against *E. coli* when compared with standard ciprofloxacin (MIC = 25.00 μg/mL). The compound **5d** (MIC = 28.50 μg/mL) had shown comparable activity with that of ciprofloxacin against *E. coli* strain. All other compounds **5b** (MIC = 39.58 μg/mL), **5c** (MIC = 33.58 μg/mL), **5e** (MIC = 40.91 μg/mL), **5f** (MIC = 45.58 μg/mL), **5g** (MIC = 32.58 μg/mL), **5h** (MIC = 35.50 μg/mL), **5i** (MIC = 42.50 μg/mL), **5k** (MIC = 36.50 μg/mL), **5l** (MIC = 35.25 μg/mL) and **5m** (MIC = 37.33 μg/mL) were less active than ciprofloxacin against *E. coli*. However, all the synthesized compounds **5**(**a**–**m**) (MIC range = 7.66–36.25 μg/mL) had shown better antibacterial activity against *B. subtilis* when compared with standard ciprofloxacin (MIC = 50.00 μg/mL). Compounds **5a** and **5j** showed broad spectrum activity active against both Gram-positive and Gram-negative bacteria.

SAR studies for antibacterial activity revealed that compounds with *R = phenyl* **5a** or *R = pyrazinyl* **5j** is good for antibacterial activity. The replacement of H atom of phenyl ring by *2-OH* **5b** or *4-OH* **5c** had resulted in decrease in antibacterial activity by 2–3 folds. When *2-OH* group **5b** of phenyl ring was replaced *2-NO_2* **5d** resulted in increase in antibacterial activity. But replacement of *2-NO_2* **5d** with *3-NO_2* **5e** led to decrease in antibacterial activity. Further, introduction of *4-Cl* on 2-nitrophenyl ring **5f** led to decrease in activity by 1.5 fold. Replacement of *4-OH* **5c** with *4-COOH* **5g** on phenyl ring has led no change in antibacterial activity. When *4-COOH* **5g** group on phenyl ring was replaced with *4-CF_3* **5h** led to decrease in antibacterial activity. Replacement of *4-CF_3* **5h** with *3-CF_3* **5i** on phenyl led to further decrease in antibacterial activity. This suggested that compounds with no substitution or electron-donating groups at 4th position of phenyl ring (head group) showed better activity. When phenyl/substituted phenyl ring was replaced by heterocyclic rings like 1,2,4-triazolyl **5k**, 6-methoxybenzothiazolyl **5l** and 3-fluoro-4-morpholinophenyl **5m**, the antibacterial activity was not altered significantly.

In order to gain more insight on the binding mode of the compounds with *Peptide deformylase* (PDF), we docked the synthesized

Table 2
Docking statistics of synthesized compounds **5**(**a**–**m**) against *E. coli* PDF-Ni

Entry	Affinity (kcal/mol)	H-bonds	H-bonding ligand		H-binding receptor			H-bonds length (Å)
			Element	Atom No.	Residue	Element	Atom No.	
5a	−70.48	03	N of CN	14	GLY89	H	1414	1.62
			N of NH	16	ARG97	H	1546	2.36
			H of NH	33	ARG97	N	1533	2.17
5b	−52.06	02	O of OH	23	ARG97	H	1545	2.01
			H of OH	39	ARG97	N	1533	2.10
5c	−53.68	01	N of CN	14	ARG97	H	1545	2.22
5d	−65.38	01	N of NH	16	ARG97	H	1546	2.34
5e	−50.48	03	N of CN	14	GLY89	H	1414	2.12
			N of NH	16	ARG97	H	1546	2.33
			H of NH	36	ARG97	H	1533	2.44
5f	−60.88	03	N of NH	16	ARG97	H	1546	1.79
			N of NO_2	24	ARG97	H	1546	2.09
			O of NO_2	25	ARG97	H	1545	1.93
5g	−57.95	01	O of COOH	25	CYS129	H	2053	2.25
5h	−57.18	03	N of CN	14	ARG97	H	1544	2.16
			N of NH	16	ARG97	H	1546	1.75
			H of NH	37	ARG97	N	1533	1.70
5i	−61.02	02	F of CF_3	25	ILE44	H	707	1.51
			F of CF_3	25	GLY45	H	722	2.23
5j	−63.68	01	N of CN	14	GLY89	H	1414	1.95
5k	−58.04	01	N of NH	16	GLY89	H	1414	2.37
5l	−57.44	02	N of NH	16	ARG97	H	1546	2.43
			S	21	ARG97	H	1546	2.03
5m	−54.54	01	F	29	ILE44	H	707	1.87

compounds **5**(**a**–**m**) against crystal structure of *E. coli* PDF-Ni (PDB ID: 1G2A) which is obtained Protein Data Bank. The standard operating procedure implemented in VLife MDS 4.3 package was followed for GRIP batch docking of final synthesized compounds against three-dimensional structures of *E. coli* PDF-Ni enzyme. Docking calculation and hydrogen bond interactions are shown in Table 2. The interaction energy of the compounds **5**(**a**–**m**) and their PDF inhibition activity showed the corresponding results. The active compounds **5a**, **5d** and **5j** showed lowest interaction energy that is −70.48 kcal/mol, −65.38 kcal/mol and −63.68 kcal/mol, respectively. The docking results indicated that of these compounds **5**(**a**–**m**) held in the active pocket by combination of hydrophobic and van der Waals interactions with the PDF enzyme. The various hydrophobic and van der Waal's interactions occurred between these compounds and active site of PDF enzyme include GLU41, GLU42, GLY43, ILE44, GLY45, ILE86, GLU87, GLY88, GLY89, CYS90, LEU91, PRO94, GLU95, ARG97, LEU125, ILE128, CYS129, HIS132 and GLU133.

The docking interactions of most active compounds **5a**, **5d** and **5j** against *E. coli* PDF-Ni is shown in Figure 2. Compound **5a** had shown good binding interactions with amino acids and held in active pocket by forming various hydrophobic and van der Waal's bonding with side chain of GLU41, GLU42, GLY43, ILE44, GLY45, ILE86, GLU88, GLY89, GLY90, LEU91, ARG97, LEU125, CYS129 and GLU133. The amino acids GLY89 (1.62 Å), ARG97 (2.36 Å) and ARG97 (2.17 Å) had shown hydrogen bonding interactions with nitrogen of –CN, nitrogen of –NH and hydrogen of –NH, respectively with compound **5a**. The compound **5d** was also held in active pocket of enzyme by forming various interactions with amino acids residues like GLU42, GLY43, ILE44, GLY45, GLY89, CYS90, LEU91, GLU95, ARG97, CYS129, HIS132 and GLU133. The nitrogen atom of –NH had formed hydrogen bond with amino acid ARG97 (2.34 Å). The nitro group was held in active pocket of enzyme by forming van der Waal's interaction with amino acid ARG97. The docking study of compound **5j** revealed that compound is buried deep into the active site by forming various hydrophobic and van der Waal's interactions with amino acid residues like, GLU41, GLU42, GLY43, ILE44, GLU88, GLY89, CYS90, LEU91, ARG97, CYS129 and GLU133. The amino acid had GLY89 (1.95 Å) had formed strong hydrogen bonds with nitrogen of –CN group. On the basis of activity data and docking result, it was found the compounds **5a**, **5d** and **5j** had potential to inhibit PDF enzyme.

Many potential therapeutic agents fail to reach the clinical stage because of their unfavourable absorption, distribution, metabolism, and elimination (ADME) parameters. Therefore, a computational study of synthesized compounds **5**(**a**–**m**) was performed for assessment of ADME properties and value obtained is depicted in Table 3. Polar surface area (TPSA), number of rotatable bonds (*n*-ROTB), molecular volume (MV), and Lipinski's rule of five were calculated using Molinspiration online property calculation toolkit. From all these parameters, it can be observed that all the synthesized compounds exhibited excellent % absorption (86.56–97.16%). The most active compounds **5a**, **5d** and **5j** showed 97.16%, 97.16% and 88.27% absorption, respectively. Furthermore, these active compounds **5a**, **5d** and **5j** had not violated Lipinski's rule of five and thus showing possible utility for developing the compound with good drug like properties. A molecule likely to be developed as an orally active drug candidate should show no more than one violation of the following four criteria: log*P* (octanol–water partition coefficient) $\leqslant 5$, molecular weight $\leqslant 500$, number of hydrogen bond acceptors $\leqslant 10$ and number of hydrogen bond donors $\leqslant 5$.[17] All the synthesized compounds followed the criteria for orally active drug and therefore, these compounds can be further developed as oral drug candidates. The results of this in silico ADME prediction analysis suggest that the synthesized compounds follow the computational assessment and thus represent a pharmacologically active framework that should be considered on progressing further potential hits.

3. Conclusion

In conclusion, a series of cyano substituted biaryl analogs **5**(**a**–**m**) was designed and synthesized efficiently in good yields. The synthesized compounds were evaluated for PDF inhibition and antibacterial activities. The compounds **5a** (IC_{50} = 13.16 μM), **5d** (IC_{50} = 15.66 μM) and **5j** (IC_{50} = 19.66 μM) showed promising PDF inhibition activity. Also, these active compounds **5a** (MIC range = 11.00–15.83 μg/mL), **5d** (MIC range = 23.55–28.50 μg/mL)

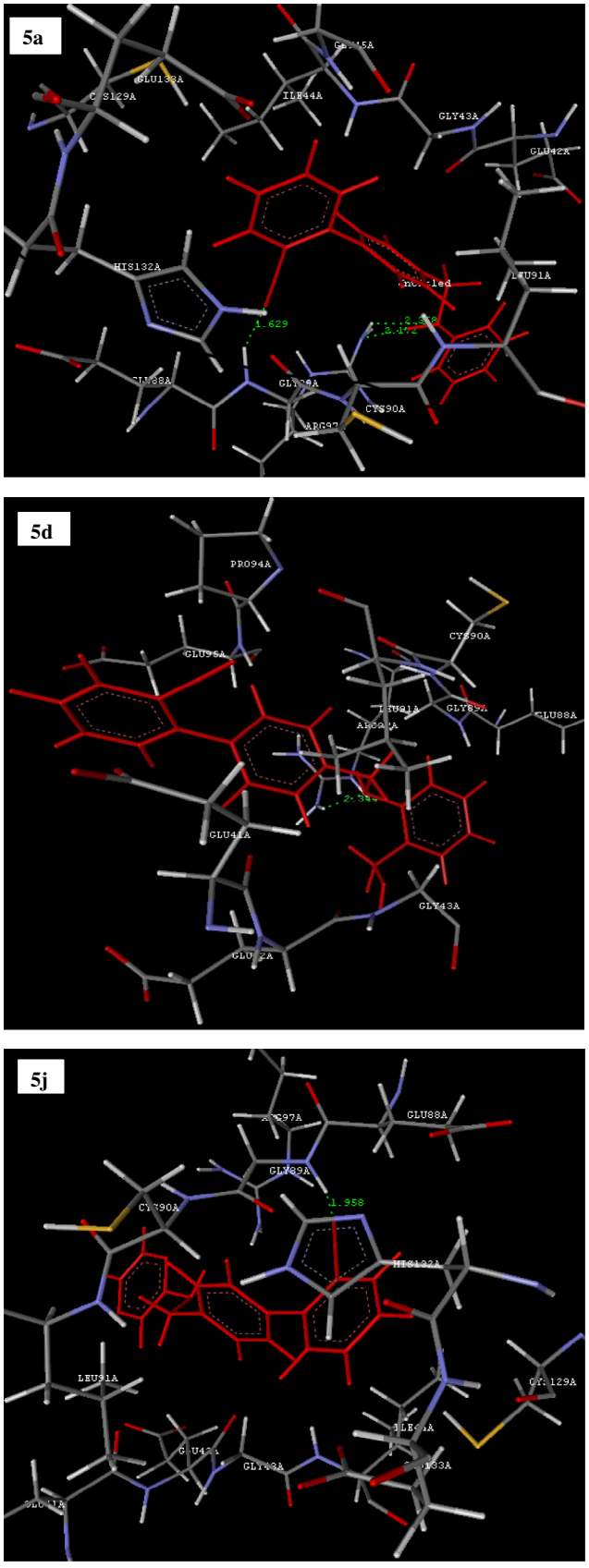


Figure 2. Docking study of compounds **5a**, **5d** and **5j** with *E. coli* PDF-Ni (PDB ID: 1G2A). Ligands are shown in red color. Hydrogen bonds are shown in green color.

and **5j** (MIC range = 7.66–16.91 μg/mL) had shown potent antibacterial activity when compared with standard ciprofloxacin (MIC range = 25–50 μg/mL). Further, to understand the mechanism of PDF inhibition, we docked the synthesized compounds **5**(**a**–**m**) against *E. coli* PDF-Ni enzyme and result suggested good binding interactions. The compounds **5a** (−70.48 kcal/mol), **5d** (−65.38 kcal/mol) and **5j** (−63.68 kcal/mol) exhibited the lowest binding energy than the remaining analogs. In other words, they possess the highest potential binding affinity into the binding site of the 3D structure of PDF enzyme. In silico ADME prediction of synthesized library indicated that compounds had potential to develop as good oral drug candidate. These findings provide important information for the exploration of compounds **5a**, **5d** and **5j** as good oral drug-like PDF inhibitors as novel antibacterial agents.

4. Experimental

4.1. Chemistry

All reagents and solvents used were obtained from the Sigma and Avra synthesis. The completion of reaction was checked by ascending thin layer chromatography (TLC) on silica gel-G (Merck) coated aluminum plates, visualized by iodine vapor. Melting points were determined by open capillary using Buchi 530 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded for the compounds on JASCO FTIR (PS 4000) using KBr pallet. ^{1}H NMR spectra were recorded on Bruker Avance II (400 MHz) using TMS (Tetramethylsilane) as the internal standard. Chemical shift values are expressed as parts per million (ppm) downfield from TMS and *J* values are in hertz. Multiplicities are recorded as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and b (broad). ^{13}C NMR spectra were recorded on Bruker Avance II (100 MHz) using TMS as internal standard. Mass spectra were taken with Micromass-QUATTRO-II of WATER mass spectrometer. Elemental analyses (C, H, and N) were undertaken with a Shimadzu's FLASHEA112 analyzer.

4.1.1. Procedure for synthesis of cyano substituted biaryl analogs 5(a–m)

Equimolar quantities of 4′-(bromomethyl)biphenyl-2-carbonitrile **4** (0.03 mol) and different substituted aromatic/heterocyclic amines (0.03 mol) were refluxed in *N*,*N*-dimethylformamide (20 mL) for 4–6 h, in presence of K_2CO_3 (0.06 mol) as a catalyst. After completion of reaction (monitored by TLC), mixture was poured into ice-water (25 mL) to obtained solid product. The solid product formed was filtered, dried and recrystallized from ethanol. All the derivatives **5**(**a**–**m**) were prepared similarly by treating with corresponding amines.

4.1.1.1. 4′-((Phenylamino)methyl)biphenyl-2-carbonitrile (5a). Yield: 84%; mp: 94–96 °C; IR (KBr, ν_{max} in cm^{-1}): 3300 (N–H), 3030 (C–H of aromatic), 2852 (C–H of CH_2), 2260 (CN); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 4.77 (s, 2H, CH_2), 5.02 (s, 1H, NH), 7.26–8.03 (m, 13H, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 47.03, 111.73, 111.78, 113.78, 118.65, 119.36, 126.84, 127.62, 128.32, 129.34, 130.69, 133.03, 137.20, 137.29, 139.03, 145.01, 151.46, 151.81, 166.53; ES-MS *m*/*z*: 285.23 [M+H$^+$]; Elemental Analysis for $C_{20}H_{16}N_2$. Calcd C, 84.48; H, 5.67; N, 9.85; Found: C, 84.30; H, 5.65; N, 9.83.

4.1.1.2. 4′-((2-Hydroxyphenylamino)methyl)biphenyl-2-carbonitrile (5b). Yield: 86%; mp: 112–114 °C; IR (KBr, ν_{max} in cm^{-1}): 3520 (O–H), 3325 (N–H), 3025 (C–H of aromatic), 2830

Table 3
In silico physicochemical pharmacokinetic parameters important for good oral bioavailability of synthesized compounds **5**(**a**–**m**)

Entry	% ABS	TPSA (A^2)	*n*-ROTB	MV	MW	miLog*P*	*n*-ON acceptors	*n*-OHNH donors	Lipinski's violations
Rule	—	—	—	—	<500	⩽5	<10	<5	⩽1
5a	97.16	34.30	4	261.85	272.35	4.88	2	1	0
5b	90.19	54.52	4	269.87	288.35	4.61	3	2	0
5c	90.19	54.52	4	269.87	288.35	4.40	3	2	0
5d	97.16	34.30	4	271.54	271.34	3.96	2	1	0
5e	97.16	34.30	4	271.54	271.34	3.98	2	1	0
5f	97.16	34.30	4	285.08	305.79	4.61	2	1	0
5g	91.28	51.36	4	267.13	287.34	4.59	3	1	0
5h	97.16	34.30	5	293.15	340.35	5.77	2	1	1
5i	97.16	34.30	5	293.15	340.35	5.75	2	1	1
5j	88.27	60.08	4	253.54	274.33	3.30	4	1	0
5k	86.56	65.02	4	238.91	263.30	2.30	5	1	0
5l	89.53	56.42	5	317.94	359.45	5.47	4	1	1
5m	92.86	46.77	5	344.92	375.45	4.92	4	1	0

% ABS: percentage absorption, TPSA: topological polar surface area, *n*-ROTB: number of rotatable bonds, MV: molecular volume, MW: molecular weight, miLog*P*: logarithm of partition coefficient of compound between *n*-octanol and water, *n*-ON acceptors: number of hydrogen bond acceptors, *n*-OHNH donors: number of hydrogen bonds donors.

(C–H of CH_2), 2215 (CN); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 4.49 (s, 2H, CH_2), 5.20 (s, 1H, NH), 5.76 (s, 1H, OH), 7.46–8.41 (m, 12H, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 48.37, 110.69, 113.21, 118.68, 119.13, 127.63, 127.87, 129.10, 129.98, 133.92, 138.49, 141.49, 144.96, 146.47, 156.49; ES-MS *m*/*z*: 301.16 [M+H$^+$]; Elemental Analysis for $C_{20}H_{16}N_2O$. Calcd C, 79.98; H, 5.37; N, 9.33; O, 5.33; Found: C, 79.30; H, 5.35; N, 9.32; O, 5.30.

4.1.1.3. 4′-((4-Hydroxyphenylamino)methyl)biphenyl-2-carbonitrile (5c). Yield: 90%; mp: 136–138 °C; IR (KBr, ν_{max} in cm^{-1}): 3536 (O–H), 3319 (N–H), 3018 (C–H of aromatic), 2823 (C–H of CH_2), 2210 (CN); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 4.26 (s, 2H, CH_2), 4.57 (s, 1H, NH), 5.63 (s, 1H, OH), 7.84–8.74 (m, 12H, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 46.33, 108.58, 110.23, 115.52, 117.69, 125.47, 126.10, 127.35, 128.59, 135.49, 137.78, 140.53, 142.25, 149.35, 158.65; ES-MS *m*/*z*: 301.25 [M+H$^+$]; Elemental Analysis for $C_{20}H_{16}N_2O$. Calcd C, 79.98; H, 5.37; N, 9.33; O, 5.33; Found: C, 79.95; H, 5.36; N, 9.31; O, 5.34.

4.1.1.4. 4′-((2-Nitrophenylamino)methyl)biphenyl-2-carbonitrile (5d). Yield: 89%; mp: 120–122 °C; IR (KBr, ν_{max} in cm^{-1}): 3330 (N–H), 3033 (C–H of aromatic), 2840 (C–H of CH_2), 2225 (CN), 1550 (N–O); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 5.23 (s, 2H, CH_2), 5.82 (s, 1H, NH), 7.00–7.82 (m, 12H, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 40.70, 111.24, 115.08, 118.63, 120.82, 125.75, 127.22, 129.12, 132.88, 134.11, 136.19, 138.04, 144.93, 151.82; ES-MS *m*/*z*: 330.15 [M+H$^+$]; Elemental Analysis for $C_{20}H_{15}N_3O_2$. Calcd C, 72.94; H, 4.59; N, 12.76; O, 9.72; Found: C, 72.90; H, 4.59; N, 12.74; O, 9.70.

4.1.1.5. 4′-((3-Nitrophenylamino)methyl)biphenyl-2-carbonitrile (5e). Yield: 89%; mp: 146–148 °C; IR (KBr, ν_{max} in cm^{-1}): 3335 (N–H), 3040 (C–H of aromatic), 2832 (C–H of CH_2), 2220 (CN), 1542 (N–O); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 4.40 (s, 2H, CH_2), 5.43 (s, 1H, NH), 6.83–7.71 (m, 12H, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 47.71, 106.59, 111.24, 112.33, 118.74, 127.66, 127.77, 129.84, 130.00, 132.88, 133.76, 138.71, 144.98, 148.64; ES-MS *m*/*z*: 330.34 [M+H$^+$]; Elemental Analysis for $C_{20}H_{15}N_3O_2$. Calcd C, 72.94; H, 4.59; N, 12.76; O, 9.72; Found: C, 72.98; H, 4.58; N, 12.78; O, 9.75.

4.1.1.6. 4′-((4-Chloro-2-nitrophenylamino)methyl)biphenyl-2-carbonitrile (5f). Yield: 88%; mp: 140–142 °C; IR (KBr, ν_{max} in cm^{-1}): 3335 (N–H), 3040 (C–H of aromatic), 2832 (C–H of CH_2), 2220 (CN), 1542 (N–O), 850 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 4.78 (s, 2H, CH_2), 5.22 (s, 1H, NH), 7.45–7.78 (m, 11H, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 48.27, 111.25, 118.69, 127.22, 128.94, 130.01, 132.81, 133.73, 133.92, 137.42, 141.48, 145.19, 154.41; ES-MS *m*/*z*: 364.08 [M+H$^+$]; Elemental Analysis for $C_{20}H_{14}ClN_3O_2$. Calcd C, 66.03; H, 3.88; Cl, 9.75; N, 11.55; O, 8.80; Found: C, 65.86; H, 3.88; Cl, 9.72; N, 11.52; O, 8.82.

4.1.1.7. 4′-((4-Carboxyphenylamino)methyl)biphenyl-2-carbonitrile (5g). Yield: 82%; mp: 172–174 °C; IR (KBr, ν_{max} in cm^{-1}): 3348 (N–H), 3260 (O–H of COOH), 3013 (C–H of aromatic), 2815 (C–H of CH_2), 2232 (CN), 1760 (C═O of COOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 5.09 (s, 2H, CH_2), 5.64 (s, 1H, NH), 7.24–7.67 (m, 12H, aromatic), 10.01 (s, 1H, COOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 47.24, 111.19, 113.78, 118.65, 119.36, 127.17, 127.62, 128.21, 128.90, 131.79, 133.73, 137.20, 137.29, 139.03, 145.01, 151.04, 151.81, 166.49; ES-MS *m*/*z*: 329.35 [M+H$^+$]; Elemental Analysis for $C_{21}H_{16}N_2O_2$. Calcd C, 76.81; H, 4.91; N, 8.53; O, 9.74; Found: C, 75.86; H, 4.88; N, 8.50; O, 9.72.

4.1.1.8. 4′-((4-(Trifluoromethyl)phenylamino)methyl)biphenyl-2-carbonitrile (5h). Yield: 90%; mp: 160–162 °C; IR (KBr, ν_{max} in cm^{-1}): 3346 (N–H), 3013 (C–H of aromatic), 2815 (C–H of CH_2), 2232 (CN), 1115 (C–F); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 4.44 (s, 2H, CH_2), 4.80 (s, 1H, NH), 6.64–7.77 (m, 12H, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 47.41, 111.20, 112.00, 118.71, 126.62, 126.67, 127.62, 129.15, 129.97, 132.86, 133.74, 137.36, 139.15, 145.01, 150.34; ES-MS *m*/*z*: 353.40 [M+H$^+$]; Elemental Analysis for $C_{21}H_{15}F_3N_2$. Calcd C, 71.58; H, 4.29; F, 16.18; N, 7.95; Found: C, 70.96; H, 4.29; F, 15.96; N, 7.93.

4.1.1.9. 4′-((3-(Trifluoromethyl)phenylamino)methyl)biphenyl-2-carbonitrile (5i). Yield: 85%; mp: 154–156 °C; IR (KBr, ν_{max} in cm^{-1}): 3350 (N–H), 3075 (C–H of aromatic), 2836 (C–H of CH_2), 2218 (CN), 1122 (C–F); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 4.42 (s, 2H, CH_2), 4.77 (s, 1H, NH), 6.77–7.77 (m, 12H, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 47.74, 109.07, 111.23, 114.05, 114.08, 115.71, 118.71, 126.98, 127.60, 127.78, 129.15, 129.72, 132.84, 133.74, 137.36, 139.27, 145.05, 148.10; ES-MS *m*/*z*: 353.32 [M+H$^+$]; Elemental Analysis for $C_{21}H_{15}F_3N_2$. Calcd C, 71.58; H, 4.29; F, 16.18; N, 7.95; Found: C, 71.32; H, 4.28; F, 16.00; N, 7.96.

4.1.1.10. 4′-((Pyrazin-2-ylamino)methyl)biphenyl-2-carbonitrile (5j). Yield: 88%; mp: 110–112 °C; IR (KBr, ν_{max} in cm^{-1}): 3362 (N–H), 3063 (C–H of aromatic), 2820 (C–H of CH_2), 2228 (CN), 1335 (C–N of pyrazin); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 4.74 (s, 2H, CH_2), 5.02 (s, 1H, NH), 7.24–7.74 (m, 11H, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 44.44, 105.37, 111.14, 127.18, 127.52, 128.23, 128.89, 133.68, 141.86, 144.96, 162.51;

ES-MS *m*/*z*: 287.35 [M+H$^+$]; Elemental Analysis for $C_{18}H_{14}N_4$. Calcd C, 75.50; H, 4.93; N, 19.57; Found: C, 74.88; H, 4.92; N, 19.75.

4.1.1.11. 4′-((4*H*-1,2,4-Triazol-4-ylamino)methyl)biphenyl-2-carbonitrile (5k). Yield: 85%; mp: 116–118 °C; IR (KBr, ν_{max} in cm^{-1}): 3355 (N–H), 3058 (C–H of aromatic), 2835 (C–H of CH_2), 2217 (CN), 1324 (C–N of tetrazole); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 4.69 (s, 2H, CH_2), 5.87 (s, 1H, NH), 7.19–7.71 (m, 10H, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 43.75, 110.04, 110.10, 110.21, 110.24, 126.22, 127.91, 129.02, 131.84, 132.70, 132.75, 132.96, 150.70; ES-MS *m*/*z*: 276.35 [M+H$^+$]; Elemental Analysis for $C_{16}H_{13}N_5$. Calcd C, 69.80; H, 4.76; N, 25.44; Found: C, 70.25; H, 4.75; N, 25.60.

4.1.1.12. 4′-((6-Methoxybenzothiazol-2-ylamino)methyl)biphenyl-2-carbonitrile (5l). Yield: 84%; mp: 154–156 °C; IR (KBr, ν_{max} in cm^{-1}): 3340 (N–H), 3018 (C–H of aromatic), 2843 (C–H of CH_2), 2239 (CN), 1220 (C–O); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.80 (s, 3H, OCH_3), 4.69 (s, 2H, CH_2), 5.74 (s, 1H, NH), 7.41–7.52 (m, 11H, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 48.70, 55.86, 105.37, 111.01, 111.04, 113.49, 118.68, 119.37, 119.54, 127.13, 127.19, 127.97, 129.10, 129.98, 133.06, 133.69, 138.49, 141.86, 144.96, 145.47, 156.21, 165.55; ES-MS *m*/*z*: 372.22 [M+H$^+$]; Elemental Analysis for $C_{22}H_{17}N_3OS$. Calcd C, 71.14; H, 4.61; N, 11.31; O, 4.31; S, 8.63; Found: C, 72.20; H, 4.60; N, 11.35; O, 4.31; S, 8.60.

4.1.1.13. 4′-((3-Fluoro-4-morpholinophenylamino)methyl) biphenyl-2-carbonitrile (5m). Yield: 80%; mp: 168–170 °C; IR (KBr, ν_{max} in cm^{-1}): 3329 (N–H), 3015 (C–H of aromatic), 2820 (C–H of CH_2), 2240 (CN), 1100 (C–F); ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.77 (t, *j* = 7.1 Hz, 4H, $2CH_2$), 4.27 (t, *j* = 7.1 Hz, 4H, $2CH_2$), 4.61 (s, 2H, CH_2), 5.47 (s, 1H, NH), 7.42–7.47 (m, 11H, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 47.23, 50.80, 53.35, 100.68, 107.32, 110.21, 119.38, 119.41, 126.04, 126.56, 126.72, 128.06, 131.84, 132.75, 136.18, 138.80, 144.11, 155.07, 157.01; ES-MS *m*/*z*: 388.19 [M+H$^+$]; Elemental Analysis for $C_{24}H_{22}FN_3O$. Calcd C, 74.40; H, 5.72; F, 4.90; N, 10.85; O, 4.13; Found: C, 75.32; H, 5.75; F, 4.90; N, 10.32; O, 4.12.

4.2. Biological evaluations

4.2.1. In vitro PDF inhibition activity

The enzyme was extracted from *Escherichia coli* (NCIM-2931). The cells are grown in LB media medium at 37 °C for 24 h. After 24 h, cells were collected by centrifugation at 10,000*g* for 10 min, and subjected to enzyme extractions. For the extractions of enzyme, cells were lyzed in phosphate buffer (0.1 M, pH 7.5, containing 5 mM $NiCl_2$) by sonication method (3 min, at 30% amplitude, with cycle interval 2 s). After sonication, the cytoplasm content was centrifugation at 12,000*g* at 10 °C for 30 min and the supernatant was used as a crude extract for the assay of the *Peptide deformylase* inhibition activity.

Peptide deformylase inhibition activity was determined through a spectrophotometric assay. Briefly, in a total of 50 μL of reaction volume with 250 μg of crude protein in buffer (100 mM phosphate buffer, pH 7.4, containing 100 μg/mL catalase) was incubated with the substrate (0-40 mM *N*-formyl-Met-Ala) at 30 °C for 30 min. The reaction was terminated by the addition of 50 μL of 4% $HClO_4$ and further incubated (30 °C for 2 h) with 2,4,6-trinitrobenzene sulfonic acid reagent (0.01% in 0.1 M $NaHCO_3$ buffer, pH 8.4). Following the addition of 10% SDS (250 μL) and 1 M HCl (125 μL), the highly chromogenic derivative generated due to reaction of primary amine with 2,4,6-trinitrobenzene sulfonic acid and monitored through measurement of the absorption at 335 nm. The values obtained were corrected by subtracting the blank (all ingredients except enzyme) readings. The specific enzyme activity was calculated from standard curves prepared with methionine (0–100 nM) and expressed as nM of free amino group produced/min/mg of protein.[18]

4.2.2. In vitro antibacterial activity

All the synthesized compounds were screened for in vitro antibacterial activity. The antibacterial activity was evaluated against two different bacterial strains such as *E. coli* (NCIM-2256) and *B. subtilis* (NCIM-2063). Dimethyl sulfoxide (DMSO) was used as solvent control. Minimum inhibitory concentration (MIC) values were determined using method recommended by National Committee for Clinical Laboratory Standards (NCCLS).[19] Ciprofloxacin and were used as a standard for the comparison of antibacterial activity.

In vitro antibacterial activities of the synthesized compounds **5** (**a**–**m**) were tested in Nutrient broth (NB) for bacteria by the two fold serial dilution method. Seeded broth (broth containing microbial spores) was prepared in NB from 24 h old bacterial cultures on nutrient agar (Hi-media) at 37 ± 1 °C. The bacterial suspension was adjusted with sterile saline to a concentration of 1×10^{-4}–10^{-5} CFU. The synthesized compounds and standard drugs were prepared by two fold serial dilutions to obtain the required concentrations of 100, 50, 25, 12.5, 6.25, and 3.13 μg/mL. The tubes were incubated in BOD incubators at 37 ± 1 °C for bacteria. The MICs were recorded by visual observations after 24 h (for bacteria) of incubation.

4.3. Computational studies

4.3.1. Molecular docking study

Molecular docking study was performed using VLife MDS 4.3 package. With this purpose, crystal structure of *Peptide deformylase* of *E. coli* (PDB ID: 1G2A)[20] was obtained from the Protein Data Bank in order to prepare protein for docking study. Docking procedure was followed using the standard protocol implemented in VLife MDS 4.3[21] package and the compounds were docked against three dimensional structure of *E. coli* PDF-Ni enzyme.

4.3.2. In silico ADME prediction

A computational study of synthesized compounds **5**(**a**–**m**) was performed for prediction of ADME properties. In this study, we calculated molecular volume (MV), molecular weight (MW), logarithm of partition coefficient (miLog*P*), number of hydrogen bond acceptors (*n*-ON), number of hydrogen bonds donors (*n*-OHNH), topological polar surface area (TPSA), number of rotatable bonds (*n*-ROTB) and Lipinski's rule of five[22] using Molinspiration online property calculation toolkit.[23] Absorption (% ABS) was calculated by: % ABS = 109 − (0.345 × TPSA).[24]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2016.05.051.

References and notes

1. Amabile-Cuevas, C. F.; Arredondo-Garcia, J. L.; Cruz, A.; Rosas, I. *J. Appl. Microbiol.* **2010**, *108*, 158.
2. Nikaido, H.; Pages, J. M. *FEMS Microbiol. Rev.* **2012**, *36*, 340.
3. Coates, A.; Hu, Y.; Bax, R.; Page, C. *Nat. Rev. Drug Disc.* **2002**, *1*, 895.
4. (a) Monaghan, R. L.; Barrett, J. F. *Biochem. Pharmacol.* **2006**, *71*, 901; (b) Fernandes, P. *Nat. Biotechnol.* **2006**, *24*, 1499.
5. Yuan, Z.; Trias, J.; White, R. J. *Drug Discovery Today* **2001**, *6*, 954.
6. Sangshetti, J. N.; Khan, F. A. K.; Shinde, D. B. *Curr. Med. Chem.* **2015**, *22*, 214.
7. Meinnel, T.; Mechulam, Y.; Blanquet, S. *Biochimie* **1993**, *75*, 1061.
8. (a) Kelly, A.; Magdalena, Z. *Progr. Med. Chem.* **2006**, *44*, 110; (b) Boularot, A.; Giglione, C.; Petit, S.; Duroc, Y.; Alves de Sousa, R.; Larue, V.; Cresteil, T.; Dardel, F.; Artaud, I.; Meinnel, T. *J. Med. Chem.* **2007**, *50*, 10; (c) Gross, P. J.; Hartmann, C. E.; Nieger, M.; Brase, S. *J. Org. Chem.* **2010**, *75*, 229.
9. Barbara, G. G.; Jeffrey, H. T.; John, W. K.; Stephan, K. G. *Arch. Biochem. Biophys.* **2000**, *375*, 355.
10. (a) Sangshetti, J. N.; Dharmadhikari, P. P.; Chouthe, R. S.; Fatema, B.; Lad, V.; Karande, V.; Darandale, S. N.; Shinde, D. B. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2250; (b) Sangshetti, J. N.; Shaikh, R. I.; Khan, F. A. K.; Patil, R. H.; Marathe, S. D.; Gade, W. N.; Shinde, D. B. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1605; (c) Sangshetti, J. N.; Khan, F. A. K.; Chouthe, R. S.; Damale, M. G.; Shinde, D. B. *Chin. Chem. Lett.* **2014**, *25*, 1033.
11. (a) Sangshetti, J. N.; Chabukswar, A. R.; Shinde, D. B. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 444; (b) Zaheer, Z.; Khan, F. A. K.; Sangshetti, J. N.; Patil, R. H. *EXCLI J.* **2015**, *14*, 935.
12. (a) Zaheer, Z.; Khan, F. A. K.; Sangshetti, J. N.; Patil, R. H. *Chin. Chem. Lett.* **2015**. http://dx.doi.org/10.1016/j.cclet.2015.10.028; (b) Sangshetti, J. N.; Khan, F. A. K.; Patil, R. H.; Marathe, S. D.; Gade, W. N.; Shinde, D. B. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 874.
13. (a) Sangshetti, J. N.; Shinde, D. B. *Eur. J. Med. Chem.* **2011**, *46*, 1040; (b) Sangshetti, J. N.; Khan, F. A. K.; Kulkarni, A. A.; Patil, R. H.; Pachpinde, A. M.; Lohar, K. S.; Shinde, D. B. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 829.
14. (a) Khan, F. A. K.; Sangshetti, J. N. *Int. J. Pharm. Pharm. Sci.* **2015**, *7*, 223; (b) Sangshetti, J. N.; Lokwani, D. K.; Chouthe, R. S.; Ganure, A.; Raval, B.; Khan, F. A. K.; Shinde, D. B. *Med. Chem. Res.* **2014**, *23*, 4893.
15. (a) Sangshetti, J. N.; Khan, F. A. K.; Qazi, Y. Q.; Damale, M. G.; Zaheer, Z. *Int. J. Pharm. Pharm. Sci.* **2014**, *6*, 217; (b) Sangshetti, J. N.; Nagawade, R. R.; Shinde, D. B. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3564; (c) Sangshetti, J. N.; Shinde, D. B. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 742.
16. Kamble, R. R.; Biradar, D. B.; Meti, G. Y.; Taj, T.; Gireesh, T.; Khazi, I. A. M.; Vaidyanathan, S. T.; Mohandoss, R.; Sridhar, B.; Parthasarath, V. *J. Chem. Sci.* **2011**, *123*, 393.
17. Ertl, P.; Rohde, B.; Selzer, P. *J. Med. Chem.* **2000**, *43*, 3714.
18. Groche, D.; Becker, A.; Schlichting, I.; Kabsch, W.; Schultz, S.; Wagner, A. F. V. *Biochem. Biophys. Res. Commun.* **1998**, *246*, 342.
19. (a) Greenwood, D.; Slack, R. C. B.; Peutherer, J. F. *Medical Microbiology*; ELBS: London, 1992. Chapter 1; (b) He, X.; Reeve, A. M.; Desai, U. R.; Kellogg, G. E.; Reynolds, K. A. *Antimicrob. Agents Chemother.* **2004**, *48*, 3093.
20. Clements, J. M.; Beckett, R. P.; Brown, A.; Catllin, G.; Lobell, M.; Palan, S.; Thomas, W.; Whittaker, M.; Wood, S.; Salama, S.; Baker, P. J.; Rodgers, H. F.; Barynin, V.; Rice, D. W.; Hunter, M. G. *Antimicrob. Agents Chemother.* **2001**, *45*, 563.
21. *VLife Molecular Design Suite 4.3*; VLife Sciences Technologies Pvt. Ltd, 2016. www.Vlifesciences.com.
22. Lipinski, C. A.; Lombardo, L.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **2001**, *46*, 3.
23. Molinspiration Chemoinformatics Brastislava, Slovak Republic, Available from: http://www.molinspiration.com/cgi-bin/properties, 2014.
24. Zhao, Y.; Abraham, M. H.; Lee, J.; Hersey, A.; Luscombe, N. C.; Beck, G.; Sherborne, B.; Cooper, I. *Pharm. Res.* **2002**, *19*, 1446.